Sphingomonas cynarae sp. nov., a proteobacterium that produces an unusual type of sphingan

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Strain SPC-1^T was isolated from the phyllosphere of *Cynara cardunculus* L. var. *sylvestris* (Lamk) Fiori (wild cardoon), a Mediterranean native plant considered to be the wild ancestor of the globe artichoke and cultivated cardoon. This Gram-stain-negative, catalase-positive, oxidase-negative, non-spore-forming, rod-shaped and non-motile strain secreted copious amounts of an exopolysaccharide, formed slimy, viscous, orange-pigmented colonies and grew optimally at around pH 6.0–6.5 and 26–30 °C in the presence of 0–0.5 % NaCl. Phylogenetic analysis based on comparisons of 16S rRNA gene sequences demonstrated that SPC-1^T clustered together with species of the genus *Sphingomonas sensu stricto*. The G+C content of the DNA (66.1 mol%), the presence of Q-10 as the predominant ubiquinone, *sym*-homospermidine as the predominant polyamine, 2-hydroxymyristic acid ($C_{14:0}$ 2-OH) as the major hydroxylated fatty acid, the absence of 3-hydroxy fatty acids and the presence of sphingoglycolipid supported this taxonomic position. 16S rRNA gene sequence analysis showed that SPC-1^T was most closely related to *Sphingomonas hankookensis* ODN7^T, *Sphingomonas insulae* DS-28^T and *Sphingomonas panni* C52^T (98.19, 97.91 and 97.11 % sequence similarities, respectively). However, DNA–DNA hybridization analysis did not reveal any relatedness at the species level. Further differences were apparent in biochemical traits, and fatty acid, quinone and polyamine profiles leading us to conclude that strain SPC-1^T represents a novel species of the genus *Sphingomonas*, for which the name *Sphingomonas cynarae* sp. nov. is proposed; the type strain is SPC-1^T (=JCM 17498^T=ITEM 13494^T). A component analysis of the exopolysaccharide suggested that it represents a novel type of sphingan containing glucose, rhamnose, mannose and galactose, while glucuronic acid, which is commonly found in sphingans, was not detected.

Sphingomonads are a group of alphaproteobacteria that are widely distributed in nature, commonly isolated from many land and water habitats, as well as from plant phyllosphere and rhizosphere, clinical specimens, and other sources. Besides their ecological role, these micro-organisms have a great potential for biotechnological applications in the degradation, bioremediation and wastewater treatment of xenobiotic pollutants, as bacterial antagonists of phyto-pathogenic fungi and for the production of industrially useful polymers and enzymes (Fialho *et al.*, 2008; Lal *et al.*,

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Abbreviations: EPS, exopolysaccharide; FAME, fatty acid methyl ester; HPAEC-PAD, high-performance anion-exchange chromatography with pulsed amperometric detection; TFA, trifluoroacetic acid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SPC-1^T is HQ439186.

Two supplementary tables, five supplementary figures and two supplementary files are available with the online version of this paper.

2006; Nilgiriwala *et al.*, 2008; Stolz, 2009; White *et al.*, 1996). Here, we report the taxonomic characterization of the orange-pigmented strain SPC-1^T, which was preliminarily identified as a member of the genus *Sphingomonas*. The strain was isolated from the phyllosphere of *Cynara cardunculus* L. var. *sylvestris* (Lamk) Fiori (wild cardoon), a non-domesticated robust perennial plant characterized by a rosette of large spiny leaves and branched flowering stems considered to be the wild ancestor of the globe artichoke and cultivated cardoon. In addition, evidence is provided that strain SPC-1^T secretes copious amounts of an unusual type of sphingan that lacks uronic acids.

Strain SPC-1^T was isolated in pure culture by means of the standard dilution plating technique on trypticase soy agar (TSA). Single orange-pigmented colonies were visible after cultivation at 28 °C for 48 h. The type strains of three recognized *Sphingomonas* species were used as reference strains for DNA–DNA hybridization and phenotypic characterization: *Sphingomonas insulae* DSM 21792^T (original strain designation: DS-28^T), *Sphingomonas panni* DSM 15761^T (original strain designation: C52^T) and *Sphingomonas hankookensis* DSM 23329^T (original strain designation: ODN7^T). These strains were obtained from the DSMZ, Braunschweig, Germany. Morphological, physiological and biochemical characteristics of strain SPC-1^T with respect to reference strains, which were tested in parallel, were investigated using routine cultivation at 28 °C on TSA or trypticase soy broth (TSB). Cell morphology and motility were examined by light microscopy. Gram staining was done using the bioMérieux Gram stain kit according to the manufacturer's instructions. Temperature tolerance (4–40 °C) was examined on TSA. Salt tolerance was tested on TSA supplemented with various NaCl concentrations (0–5 %); for this, the TSA was prepared according to the formula of the Difco medium, except that NaCl was excluded from the medium formula. The pH range for growth was determined on TSA that was adjusted to various pH values (pH 4.5–10.5 at intervals of 0.5 pH units). The pH was adjusted prior to sterilization by the addition of HCl or Na_2CO_3. Catalase and oxidase activities were determined by using the ID colour Catalase (ID-ASE; bioMérieux) and the Oxidase reagent kits (bioMérieux), respectively. Utilization of various substrates, enzyme activities, and other physiological and biochemical properties were tested by using the API 20E, API 20NE and API 50CH systems (bioMérieux); utilization of various substrates was determined by inoculating the API 50CH strip with bacteria suspended in AUX medium (bioMérieux). Results were recorded after 72 h incubation at 28 °C under aerobic conditions.

High-molecular-mass genomic DNA extraction from bacteria grown in TSB to late exponential phase was carried out as described previously (Stabili *et al.*, 2008). The 16S rRNA gene was amplified and sequenced using the primer pair 5′-AGAGTTTGATCATGGCTCCAG-3′ and 5′-ACGGCTACCTTGTTACGACTT-3′. PCR conditions and nucleotide sequencing procedures were as reported previously (Vigliotta *et al.*, 2007; Stabili *et al.*, 2008). The sequence was compared with those of closely related reference organisms using the EzTaxon service (Kim *et al.*, 2012). Multiple sequence alignments were performed with CLUSTAL W (version 2.1) (Thompson *et al.*, 1994) at the Kyoto University Bioinformatic Center (http://www.genome.jp/tools/clustalw/) using the following default settings: weight matrix IUB (for DNA), gap open penalty 15, gap extension penalty 6.66. Almost complete 16S rRNA gene sequences from type strains of members of the genus *Sphingomonas* were used (Table S1 and File S1, available in IJSEM Online). The CLUSTAL W output file (File S2) was utilized to infer evolutionary trees with the PHYLO_WIN package (Galtier *et al.*, 1996) according to the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Sober, 1983) and maximum-likelihood (Felsenstein, 1981) methods. Evolutionary distances were calculated with the neighbour-joining method according to the algorithm of Kimura's two-parameter model (Kimura, 1980). Bootstrap resampling (1000 replicates each) was used to assess robustness of phylogenetic trees (Brown, 1994).

The DNA G+C content was determined using an HPLC system (Agilent 1100 Series HPLC) equipped with a Phenomenex-luna 5μ C18 (2) 100 Å column (250 × 4.6 mm). A gradient elution mode was used according to Gehrke & Kuo (1990). The elution buffer was as follows: eluent A (2.50 % methanol in 0.01 M $NH_4H_2PO_4$; pH 5.3), eluent B (20.0 % methanol in $NH_4H_2PO_4$; pH 5.1), eluent C (35 % acetonitrile in 0.01 M $NH_4H_2PO_4$; pH 4.9) at flow rate of 1 ml min^{-1} at 25 °C. Each nucleoside was detected by its UV absorbance at 270 nm. DNA was hydrolysed by nuclease P1 (Sigma) and the resultant nucleotides were treated with alkaline phosphatase (2.4 units ml^{-1}) and then analysed by reversed-phase HPLC.

DNA–DNA hybridization experiments were carried out by using the membrane filter method described by Ezaki *et al.* (1989) with modifications (Stabili *et al.*, 2008). Total DNAs (5 μg) from the different bacterial strains were restricted with *Hae*III, serially diluted in a buffer containing 2 × SSC (1 × SSC is 0.15 M NaCl plus 0.015 M sodium citrate) and 50 % (v/v) formamide, heated at 95 °C for 5 min and immobilized onto positively charged nylon filters by slow filtration in a slot-blot apparatus (Minifold I Slot-Blot System; Sigma-Aldrich) in duplicate. The filters were dried at room temperature and directly used for hybridization. DNA probes were obtained by labelling *Hae*III-restricted genomic DNAs with DIG-High Prime mix (Roche) according to manufacturer's instructions. Pre-hybridization was carried out in a buffer containing 5 × SSC, 5 × Denhardt's solution, 0.1 % SDS, 50 mM sodium phosphate buffer (pH 6.5), 50 % (v/v) formamide, 500 μg denatured salmon sperm DNA ml^{-1}. The hybridization buffer was similar to the pre-hybridization solution but containing 100 ng digoxigenin-labelled DNA per millilitre in place of salmon sperm DNA. Pre-hybridization and hybridization steps were carried out at 47 °C. This temperature represents stringent conditions for strain SPC-1^T, for which the

optimal renaturation temperature (44.7 °C) is calculated as [(0.51 × DNA G+C content)+47]−36 (Gillis *et al.*, 1970), where 36 °C is the correction for the presence of 50 % formamide (McConaughy *et al.*, 1969). A DNA G+C content of 66.1 mol% was determined for strain SPC-1^T (see below). After hybridization, filters were washed three times with a solution containing 2 × SSC, 0.2 % SDS at 47 °C. The filters were then subjected to immunological detection, according to the manufacturer's instructions. Semi-quantitative analysis of the hybridization signals was performed by densitometry using a Scanmaster 3 (Howtek), a high-performance desktop flatbed colour scanner equipped with an RFLPrint (Pdi) software package.

For analysis of respiratory quinones, polyamine pattern, polar lipids and fatty acid methyl esters (FAMEs), strain SPC-1^T and reference strains were grown to late exponential phase (to 1.5 at O.D. 600 nm) in 2 l Erlenmeyer flasks each containing 500 ml TSB at 28 °C on a rotary shaker at 200 r.p.m. in the dark. To perform respiratory quinone analysis, isoprenoid quinones were extracted from 100 mg lyophilized cell material with chloroform/methanol (2 : 1, v/v) and analysed using reversed-phase HPLC and a Phenomenex-luna 5μ C18 (2) 100 Å column (250 × 4.6 mm) according to the method of Moss & Guerrant (1983) with modifications. Mobile phases consisted of methanol (A), isopropanol (B) and water (C). The isocratic elution was as follows: 0 min, 75% A, 20% B and 5% C; 0 to 7 min, 75% A, 25% B; 7 to 32 min, 35% A, 65% B; 32 to 35 min, 65% A, 20% B and 5% C. The column was re-equilibrated for 10 min between runs. The flow rate was 1.0 ml min^{-1} and the column temperature was maintained at 25°C. The injection volume was 10 µl. Absorbance was registered by diode array at 290 nm.

Lipids were extracted using the modified method of Bligh & Dyer (1959) with some modifications. Lyophilized powder (100 mg) was mixed with a total of 114 ml solvent added in this sequence: chloroform, methanol, water to achieve a final chloroform/methanol/water ratio of 1 : 2 : 0.8 (by vol.). Samples were shaken for 15 s after addition of each solvent, and incubated overnight at 4°C. After centrifugation at 6500 × ***g*** for 10 min, the supernatant was transferred into a separating funnel, and phase separation of the biomass-solvent mixtures was achieved by adding chloroform and water to obtain a final chloroform/methanol/water ratio of 2 : 2 : 1.8 (by vol.). After settling, the bottom phase was collected. A portion of the total lipid extract was *trans*-esterified according to Eguchi *et al.* (2001) at 80 °C for 1 h using a solution of methanol/hydrochloric acid/chloroform (10 : 1 : 1). After the addition of 1 ml water, the mixture was extracted twice with 3 ml hexane/chloroform 4 : 1 (v/v) to obtain FAMEs, which were analysed using GC-MS. The GC-MS system consisted of a Shimadzu GC-17A ver. 3.0, with MS QP5050A. Compounds were separated on a DB-5 capillary column (30 m length, 0.25 mm ID and 0.25 µm thickness). The GC parameters were as follows. The temperature of the column was 80 °C after injection, then programmed at 10 °C·min^{-1} to 150 °C, at 5 °C·min^{-1} to 250 °C and maintained at that temperature for 15 min. Split injection was conducted with a split ratio of 50 : 1, the flow-rate was 1.0 ml·min^{-1}, carrier gas used was 99.999 % pure helium, the injector temperature was 250 °C and the column inlet pressure was 74 kPa. The MS detection conditions were as follows. Interface temperature was set to 250 °C; ionization mode, EI+; electron energy, 70 eV; scanning method of acquisition, ranging from 30 to 450, for mass/charge (m/z) was optimized. Spectrum data were collected at 0.5 s intervals. Solvent cut time was set at 2 min and 45 min retention time, which was sufficient for separation of all the fatty acids. Compounds were identified by using online NIST98-library spectra and published MS data. Moreover, bacterial FAME mix and PUFA-3 (from menhaden oil) authentic standards (both from Sigma-Aldrich) were used to confirm MS data and to discriminate between $C_{18:1}\omega7c$ and $C_{18:1}\omega9c$. Polar lipids were extracted according to the procedures described by Minnikin *et al.* (1984), resolved by two-dimensional TLC [first dimension, chloroform/methanol/water (65 : 25 : 4, by vol.); second dimension, chloroform/acetic acid/methanol/water (80 : 15 : 12 : 4, by vol.)] and detected by spraying with 5 % ethanolic molybdophosphoric acid followed by charring at 180°C.

Polyamines were extracted and derivatized as described by Scherer & Kneifel (1983) with some modifications. In brief, 40 mg freeze-dried samples were hydrolysed in 1 ml of 0.2 M $HClO_4$ at 100 °C for 30 min with shaking once after 15 min. Internal standard 1,8-diaminooctane (360 nmol per 40 mg cells) was added before heating and the samples were centrifuged (4500 × ***g*** for 10 min). Supernatant samples (0.2 ml) were incubated with 300 µl Na_2CO_3 solution (100 mg ml^{-1}) and 800 µl dansyl chloride (7.5 mg ml^{-1} in acetone). Proline solution (100 µl of 50 mg ml^{-1} solution) was added to bind the excess dansyl chloride during incubation at 60 °C for 10 min. After cooling at 5 °C, polyamines were extracted with 100 µl toluene. Toluene was removed under a slight stream of N_2. The volume was adjusted to 100 µl with acetonitrile and 20 µl was loaded into the HPLC system equipped with a Phenomenex-luna 5μ C18 (2) 100 Å column (250 × 4.6 mm). The mobile phase was: eluent A (70 mM acetic acid, 25 mM triethylamine, pH 4.82), eluent B (80 % acetonitrile), eluent C (methanol). A gradient elution mode was used according to the method of Shaw *et al.* (2010), at a flow rate of 1.2 ml min^{-1} at 35 °C. Detection was carried out at 254 nm. To quantify spermidine and spermine, an internal standard and a calibration curve (0.05–50 mM) were used. The presence of s*ym*-homospermidine was confirmed by comparing HPLC profiles obtained with *S. panni*.

For exopolysaccharide (EPS) production, strain SPC-1^T was cultivated for 5 days at 28 °C with rotary shaking (250 r.p.m.) in 300 ml baffled Erlenmeyer flasks with 50 ml medium containing 20 g glucose l^{-1}, 10 g peptone l^{-1}, 10 g yeast extract l^{-1} and 5 g NaCl l^{-1}. The secreted EPS material was isolated from liquid culture as described previously by West & Strohfus (1998) and Nampoothiria *et al.* (2003). Total sugar determination by the phenol-sulphuric acid

method (DuBois *et al.*, 1956) indicated that 30–40 % of the dry weight of this material comprised carbohydrates. Uronic acids were not detectable by the Blumenkrantz & Asboe-Hansen (1973) method. The dried material was hydrolysed with 2.0 M trifluoroacetic acid (TFA) in sealed tubes at 120 °C for 90 min. TFA-resistant material was precipitated by centrifugation at 8800 × ***g***, for 10 min. The supernatant (hydrolysate) was dried *in vacuo*, dissolved in 1 ml distilled water and analysed by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) as described previously by Lenucci *et al.* (2008).

Strain SPC-1^T was isolated serendipitously during investigations aimed to establish an *in vitro* propagation system for wild cardoon (Fig. S1). Morphological, physiological and biochemical characteristics of strain SPC-1^T are given in the species description and shown in Table 1. The analysis of the near-complete (1426 nt) 16S rRNA gene sequence indicated that the closest relatives of the orange-pigmented strain SPC-1^T were two members of the genus *Sphingomonas*, MK01 and PB163 (accession nos GQ339888.1 and GQ339895.1, respectively) isolated from a ginseng field and stream sediment, respectively (both showing 98.70 % sequence similarity). Among the type strains of species with validly published names, *S. hankookensis* ODN7^T (Yoon *et al.*, 2009), *S. insulae* DS-28^T (Yoon *et al.*, 2008) and *S. panni* C52^T (Busse *et al.*, 2005) showed the highest sequence similarities (98.19, 97.91 and 97.11 %, respectively). This finding was supported by phylogenetic analysis that confirmed that strain SPC-1^T belonged to the genus *Sphingomonas sensu stricto* (Takeuchi *et al.*, 2001). In the neighbour-joining phylogenetic tree, it grouped with *S. insulae* DS-28^T, *S. hankookensis* ODN7^T and *S. panni* C52^T (Fig. 1). The relationship between SPC-1^T, *S. hankookensis* ODN7^T and *S. panni* C52^T was also maintained in trees constructed via the maximum-parsimony and maximum-likelihood algorithms.

The predominant isoprenoid quinone detected in strain SPC-1^T was ubiquinone-10 (Q-10) at a peak area ratio of 83 %; minor amounts of Q-9 were also detected (Table 2, Fig. S2). This predominant quinone is typical for the members of the genus *Sphingomonas* (Yabuuchi *et al.*, 1990; Takeuchi *et al.*, 1995, 2001; Lee *et al.*, 2001; Buonaurio *et al.*, 2002; Ohta *et al.*, 2004; Busse *et al.*, 2005; Yoon *et al.*, 2008). The polyamine pattern showed the predominance of *sym*-homospermidine, the key characteristic of *Sphingomonas sensu stricto* (Busse *et al.*, 1999; Takeuchi *et al.*, 2001), with minor amounts of spermine and spermidine (Table 3, Fig. S3). Quantitative differences in both quinone and polyamine profiles between strain SPC-1^T and phylogenetically related members of the genus *Sphingomonas* could be observed (Tables 2 and 3, Figs S2 and S3).

The fatty acid profile of strain SPC-1^T was composed of the following: unsaturated fatty acids $C_{16:1}\omega7c$ (19.4 %), $C_{18:1}\omega7c$ (40.2 %), $C_{18:1}\omega9c$ (0.5 %) and $C_{22:1}\omega9$ (2.4 %); straight chain fatty acids $C_{12:0}$ (0.5 %), $C_{14:0}$ (0.8 %), $C_{16:0}$

Table 1. Differential phenotypic characteristics of strain SPC-1^T and phylogenetically related strains of other members of the genus *Sphingomonas*

Taxa: 1, strain SPC-1^T; 2, *S. insulae* DS-28^T; 3, *S. panni* C52^T; 4, *S. hankookensis* ODN7^T. All data are from the present study. Strains were growth at 28 °C on TSA. +, Positive reaction; −, negative reaction; (+), weakly positive reaction. All strains were positive for catalase, hydrolysis of aesculin and assimilation of L-arabinose, arbutin, D-glucose, 2-ketogluconate, 5-ketogluconate, lactose, D-mannose and turanose. All were negative for nitrate reduction, indole production, urease, L-arginine dihydrolase, L-lysine decarboxylase, L-ornithine decarboxylase, H_2S production and assimilation of D-adonitol, D-arabinose, D- and L-arabitol, D-dulcitol, erythritol, glycogen, inositol, inulin, D-lyxose, D-mannitol, methyl α-D-mannopyranoside, methyl β-D-xylopyranoside, D-ribose, D-sorbitol, L-sorbose, D-tagatose, L-xylose, xylitol, caprate, adipate and phenylacetate.

Characteristic	1	2	3	4
Colony pigmentation*	O	Y	Y	Y
Motility	−	+	−	−
Oxidase	−	+	−	+
β-Galactosidase	+	−	+	−
Growth in/at:				
3 % (w/v) NaCl	−	−	+	−
37 °C	−	−	+	+
pH 5.0	−	−	+	−
pH 8.0	−	+	+	+
Gelatin hydrolysis	+	−	−	−
Assimilation of:				
N-Acetylglucosamine	−	+	(+)	+
Amygdalin	−	(+)	+	+
Cellobiose	+	+	−	+
Citrate	−	+	+	+
D-Fructose	+	+	−	+
D-Fucose	−	(+)	(+)	+
L-Fucose	−	+	(+)	+
D-Galactose	+	+	−	(+)
Gentiobiose	(+)	+	−	+
Gluconate	(+)	−	−	+
Glycerol	−	+	−	−
L-Malate	−	+	+	+
Maltose	(+)	+	(+)	+
Melezitose	(+)	−	−	−
Melibiose	+	+	−	+
Methyl α-D-glucopyranoside	−	−	−	+
Raffinose	−	+	−	+
L-Rhamnose	+	+	−	+
Salicin	(+)	+	−	+
Starch	−	−	−	(+)
Sucrose	+	+	(+)	+
Trehalose	+	+	−	+
D-Xylose	−	+	+	+
Oxidative acid production from:				
D-Glucose	+	+	−	−
L-Rhamnose	+	+	−	−

*Y, Yellow; O, orange.

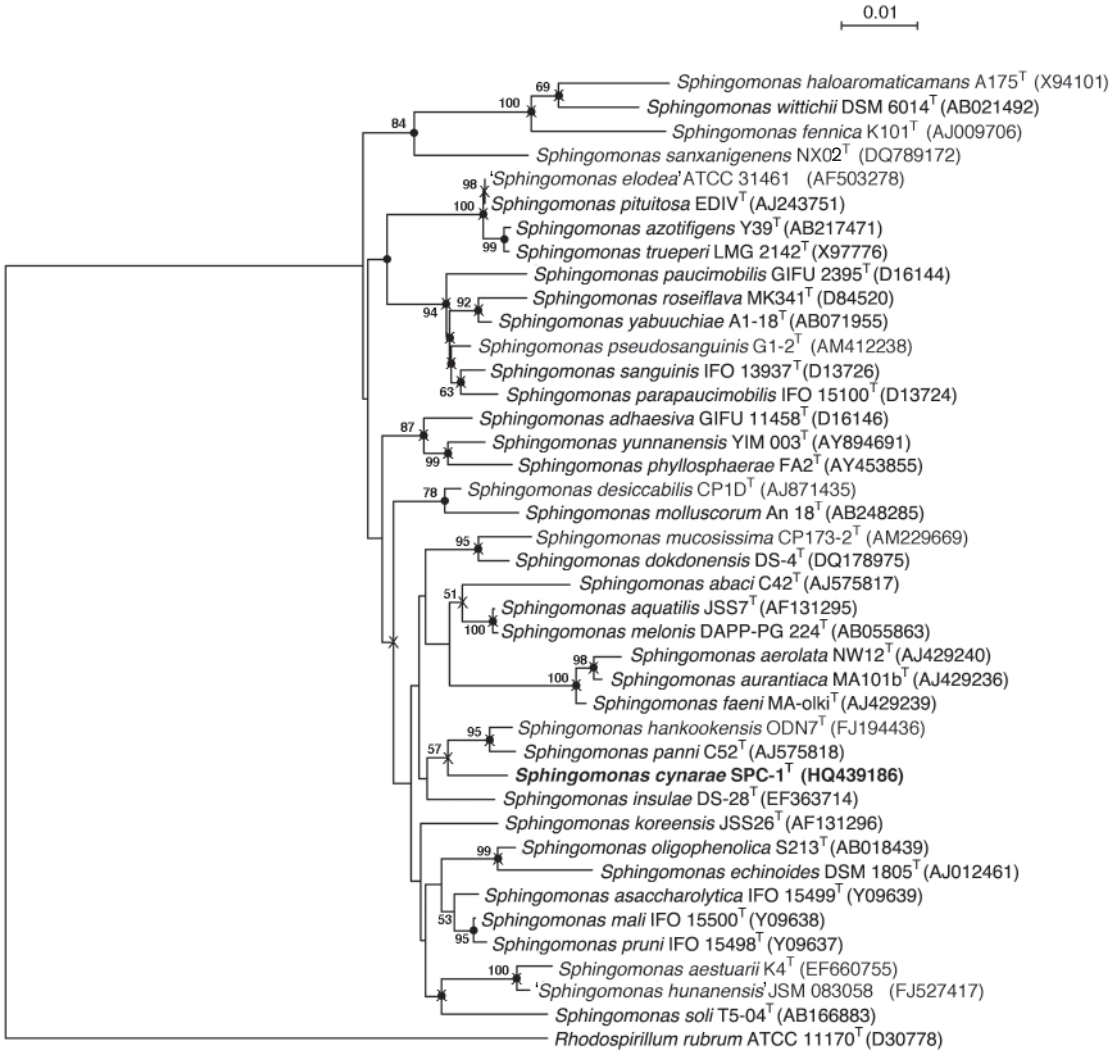

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of *Sphingomonas cynarae* sp. nov. SPC-1^T with respect to other members of the genus *Sphingomonas*. Bootstrap values (expressed as percentages of 1000 replicates) of >50 % are shown at branch points. Filled circles and 'X' indicate that the corresponding nodes were also recovered in trees generated with maximum-parsimony and maximum-likelihood algorithms, respectively. *Rhodospirillum rubrum* ATCC 11170^T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

(18.4 %), $C_{18:0}$ (8.1 %); hydroxy fatty acid $C_{14:0}$ 2-OH (5.4 %); and $C_{17:0}$ cyclo (2.0 %) (Table 4, Fig. S4). The fatty acid profile confirmed the results of phylogenetic analysis. The profile was characterized by predominance of $C_{18:1}\omega7c$ and high levels of $C_{16:0}$, which are typical of the majority of members of the alphaproteobacteria, while the presence of 2-hydroxymyristic acid ($C_{14:0}$ 2-OH) as the major hydroxylated fatty acid, and the absence of 3-hydroxy fatty acids are important markers of members of the family *Sphingomonadaceae* (Busse *et al.*, 1999, 2003; Takeuchi & Hiraishi, 2001; Takeuchi *et al.*, 2001). Quantitative differences in the fatty acid profiles between strain SPC-1^T and

Table 2. Isoprenoid quinones in strain SPC-1^T and phylogenetically related strains of other members of the genus *Sphingomonas*

Strain	Q-9 (peak area, %)	Q-10 (peak area, %)
SPC-1^T	17.0	83.0
S. insulae DS-28^T	9.7	90.3
S. panni C52^T	20.2	79.8
S. hankookensis ODN7^T	8.9	91.1

Table 3. Polyamines in strain SPC-1^T and phylogenetically related strains of other members of the genus *Sphingomonas*

Polyamine concentrations are given as μmol (g dry weight)$^{-1}$.

Strain	Spermidine	*sym*-Homospermidine	Spermine
SPC-1^T	0.84	15.88	2.71
S. insulae DS-28^T	18.63	11.81	1.04
S. panni C52^T	0.70	30.62	1.62
S. hankookensis ODN7^T	1.88	42.40	1.98

phylogenetically related members of the genus *Sphingomonas* could be also observed (Table 4). Major polar lipids identified in strain SPC-1^T were sphingoglycolipid, phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine and diphosphatidylglycerol. Small amounts of phosphatidylmonomethylethanolamine and several unidentified glyco- and phospholipids were also detected (Fig. S5). The DNA G+C content of strain SPC-1^T was 66.1 mol%.

As SPC-1^T, *S. insulae* DS-28^T, *S. hankookensis* ODN7^T and *S. panni* C52^T shared high (>97 %) 16S rRNA gene sequence similarity, quantitative DNA–DNA hybridization was used to discriminate between these taxa. Analysis of DNA relatedness between the pairs SPC-1^T/*S. insulae* DS-28^T (26.5 %, reciprocal 29.6 %), SPC-1^T/*S. hankookensis* ODN7^T (19.0 %, reciprocal 23.0 %) and SPC-1^T/*S. panni* C52^T (20.9 %, reciprocal 23.1 %) did not reveal any relatedness at the species level. This finding was consistent with the results of phenotypic analysis (Table 1). Many differential phenotypic properties could be observed between strain SPC-1^T and its close relatives *S. insulae* DS-28^T, *S. hankookensis* ODN7^T and *S. panni* C52^T, which were tested in parallel (Table 1). The highest degree of similarity in terms of physiological characteristics (about 73 %) was found between SPC-1^T and *S. insulae* DS-28^T.

Table 4. Fatty acids in strain SPC-1^T and phylogenetically related strains of other members of the genus *Sphingomonas*

Taxa: 1, strain SPC-1^T; 2, *S. insulae* DS-28^T; 3, *S. panni* C52^T; 4, *S. hankookensis* ODN7^T. Values are percentages of total fatty acids. Components representing less than 0.5 % in all strains were omitted. ND, Not detected.

Fatty acid	1	2	3	4
Saturated				
$C_{12:0}$	0.5	ND	0.6	0.5
$C_{14:0}$	0.8	3.0	2.1	2.3
$C_{16:0}$	18.4	31.0	22.9	19.8
$C_{18:0}$	8.1	5.7	3.4	4.5
Unsaturated				
$C_{16:1}\omega7c$	19.4	5.6	17.7	18.8
$C_{16:1}\omega5c$	ND	0.6	1.1	1.6
$C_{18:1}\omega7c$	40.2	40.0	42.1	42.7
$C_{18:1}\omega9c$	0.5	0.7	0.6	ND
$C_{22:1}\omega9c$	2.4	0.4	1.2	2.5
Cyclopropane				
$C_{17:0}$ cyclo	2.0	1.5	1.2	0.7
$C_{19:0}$ cyclo	ND	ND	0.5	ND
Hydroxy				
$C_{14:0}$ 2-OH	5.4	10.9	5.9	6.2

In liquid medium, strain SPC-1^T produced a considerable amount of water-soluble EPS. Analytical methods showed that SPC-1^T EPS glycosyl composition is largely different from that reported for sphingans (Fialho *et al.*, 2008) and from that we obtained from Phytagel (Sigma-Aldrich), a commercial gellan we used for comparison. HPAEC-PAD analyses revealed mannose as the most abundant sugar with a molar ratio of 42.7±4.7 %, followed by rhamnose (27.5±3.9 mol%), glucose (23.9±3.3 mol%) and galactose (5.9±1.1 mol%) (Table S2). Interestingly, glucuronic acid, the sugar conferring the anionic charge to the sphingan backbone, and galacturonic acid were not detected indicating a substantial structural diversity of the isolated SPC-1^T EPS with respect to most sphingans produced by strains of the genus *Sphingomonas* (Fialho *et al.*, 2008; White *et al.*, 1996). To our knowledge, glucuronic acid is absent only in the EPS produced by *S. paucimobilis* P4, in which the trisaccharide structure D-glucose–D-glucose–L-rhamnose is a repeating unit structure (Lobas *et al.*, 1994) and in the EPS produced by *Sphingomonas* sp. CS101 in which glucose, mannose, fucose and rhamnose were detected in a molar ratio of 2.1 : 1.1 : 1.0 : 0.1 (Seo *et al.*, 2004).

The phylogenetic distinctiveness, together with DNA–DNA relatedness data and differential phenotypic properties, was sufficient to allocate strain SPC-1^T to a species that is distinct from recognized *Sphingomonas* species (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994; Tindall *et al.*, 2010). Therefore, on the basis of the presented data, strain SPC-1^T is considered to represent a novel species of the genus *Sphingomonas*, for which the name *Sphingomonas cynarae* sp. nov. is proposed.

Description of *Sphingomonas cynarae* sp. nov.

Sphingomonas cynarae [cy.na′ra.e. N.L. gen. n. *cynarae* of *Cynara*, referring to the source of isolation *Cynara cardunculus* L. var. *sylvestris* (Lamk) Fiori (wild cardoon)].

Cells are Gram-stain-negative, non-spore-forming rods with rounded poles and are 0.4–0.5 × 1.5–3.0 μm in size. No

motility is observed by light microscopy. Colonies on TSA are convex, smooth, bright orange-pigmented and 1.5–2.0 mm in diameter after 5 days of incubation at 28 °C. Good growth occurs on TSA. Optimal temperature for growth is 28 °C. Growth does not occur at 4 °C or 37 °C. Optimal pH for growth is around 6.0–6.5. Poor growth occurs above and below this range. Growth occurs in the presence of 0–0.5 % (w/v) NaCl. Anaerobic growth does not occur on TSA or nutrient agar supplemented with nitrate. Oxidase-negative, catalase-positive. L-Arabinose, arbutin, cellobiose, D-fructose, D-galactose, gentiobiose (weakly), D-glucose, 2-ketogluconate, 5-ketogluconate, lactose, maltose (weakly), D-mannose, melibiose, melezitose (weakly), L-rhamnose, sucrose, trehalose, turanose, potassium gluconate (weakly) and salicin (weakly) are utilized as sole carbon and energy sources, but *N*-acetylglucosamine, D-adonitol, D-arabinose, D- and L-arabitol, amygdalin, D-dulcitol, erythritol, D- and L-fucose, glycerol, glycogen, inositol, inulin, D-lyxose, D-mannitol, methyl α-D-glucopyranoside, methyl α-D-mannopyranoside, methyl β-D-xylopyranoside raffinose, D-ribose, D-sorbitol, L-sorbose, starch, D-tagatose, D- and L-xylose, xylitol, trisodium citrate, capric acid, adipic acid, malic acid and phenylacetic acid are not. Negative for indole production, L-arginine dihydrolase, L-lysine decarboxylase, L-ornithine decarboxylase and urease activities, and nitrate reduction. Positive for gelatin and aesculin hydrolysis, ONPG and *p*-nitrophenyl α-D-galactopyranoside. H_2S is not produced. Susceptible to gentamicin (10 μg), ciprofloxacin (5 μg), cefuroxime (30 μg) and weakly to nitrofurantoin (100 μg), but not to chloramphenicol (30 μg) or amoxicillin/clavulanic acid (26.25/3.75 μg). The predominant ubiquinone is Q-10. The predominant polyamine is *sym*-homospermidine. The major fatty acids (>10 % of total fatty acids) are $C_{18:1}\omega7c$, $C_{16:1}\omega7c$ and $C_{16:0}$. Major polar lipids are sphingoglycolipid, phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine and diphosphatidylglycerol.

The type strain, SPC-1^T (=JCM 17498^T=ITEM 13494^T), was isolated from the phyllosphere of *Cynara cardunculus* L. var. *sylvestris* (Lamk) Fiori (wild cardoon), a Mediterranean native plant that is considered to be the wild ancestor of the globe artichoke and cultivated cardoon. The DNA G+C content of the type strain is 66.1 mol% (determined by HPLC).

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